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Prolonged rat allograft survival induced by temporary elimination of α/β T cells with monoclonal antibody

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Abstract We tested the ability of lewis (LEW; RT-1^{l}) recipients to reject DA (RT-1^{av1}) cardiac allografts following the selective elimination of α/β T cells with the mouse monoclonal antibody R 73. One group of adult rats (6 weeks old) received 1000 μg R 73 i.p. on days 2 and 1 before transplantation, and 100 μg R 73 every third day after transplantation up to day 18. Prolonged cardiac graft survival was noted (30, 30, 32, 51, 62, 108, > 500, > 500, > 500 days). Untreated controls ($n = 10$) rejected their grafts within 7 ± 1 days. R 73 therapy induced a dramatic decrease in α/β T cells from 69% before treatment to 5% within the first 5 days, followed by an increase to 64% by day 8. The T cell increase was paralleled by the appearance of anti-mouse antibody. A second group of adult rats (10 weeks old) received the same treatment. These "older" recipients rejected their grafts within 20 ± 5 days. Chronic R 73 therapy from birth until the day of transplantation (100 μg R 73 i.p. twice a week) resulted in graft survival of 37 ± 9 days in eight animals. Two rats had a graft survival of more than 200 days. When chronic R 73 therapy was continued to day 70 after transplantation, DA hearts were accepted well in all animals for more than 100 days. α/β T cells were virtually absent throughout the whole time of treatment. Antibodies against R 73 were not detected. We concluded that selective elimination of α/β T cells has a strong effect on allograft survival.

Key words Immunosuppression
α/β T cells · Tolerance induction
Heart allograft

Introduction

It has been shown that monoclonal antibodies can prolong allograft survival and even induce tolerance [1]. The most commonly studied monoclonals are directed against the T-cell receptor complex and its accompanying molecules [2]. In addition, antibodies that prevent contact between the T cell and its corresponding antigen-presenting cell by occupying various adhesion molecules have been used [3]. The monoclonal mouse antibody R 73 is directed against a constant determinant of the rat α/ TCR [4]. R 73 has been used successfully in the therap and prevention of experimental T cell dependent autoim mune diseases [5, 6]. We tested the efficacy of R 73 t prolong rat cardiac allograft survival in a strongl histoincompatible rat model.

Materials and methods

Animals

Adult Lewis LEW; (RT-1^1) and DA (RT-1^{av1}) rats were obtained from Zentralinstitut für Versuchstierzucht (Hannover, Germany). Pregnant Lewis females were purchased from Charles River WIGA (Sulzfeld, Germany). Offspring were used for R 73 therapy in newborns. All animals were kept under standard conditions.

Grafting

DA hearts were transplanted heterotopically into Lewis recipients as previously described [7]. Cardiac function was monitored by ECG and time of rejection was defined as cessation of electrophysiological activity.

Treatment

The mouse myeloma cell line R 73 (IgG_1; anti-rat-TCR α/β; Central Laboratory of the Netherlands Red Cross, Amsterdam, The Netherlands) was kindly provided by Dr. Thomas Hünig. Two different protocols of R 73 treatment were used. In the first group, adult animals received short-term R 73 treatment, whereas in a second group, chronic R 73 treatment started at the time of birth.

Short-term treatment with R 73

Group Ia Young adult Lewis rats (aged 6 weeks, weight 74 ± 5 g, $n = 9$) received 1000 µg R 73 i.p. on days -2 and -1 before, and 100 µg on the day of transplantation (day 0) and every third day thereafter until day 18.

Group Ib Older Lewis recipients (aged 10 weeks, weight 249 ± 21 g, $n = 14$) received the same regimen.

Chronic treatment with R 73

Group IIa Chronic treatment consisted of 100 µg R 73 i.p. every third day starting from birth. The treatment was stopped on the day of transplantation (age: 18 ± 5 weeks, $n = 10$).

Group IIb Chronic R 73 treatment was continued to day 70 after transplantation (age at time of transplantation 20 ± 5 weeks, $n = 10$).

Controls

Group III Control animals received no treatment ($n = 10$).

Cytofluorometry

The following antibodies were used for the determination of α/β T cells: mouse anti-rat TCR α/β (R 73, 0.4 µg) in combination with 10 µl secondary rat IgG_1 anti-mouse κ-light chain-PE antibody (X 36, Becton Dickinson, San Jose, Calif.) for indirect, and mouse IgG_1 anti-CD5-FITC (OX19, 0.6 µg; Phar-Mingen, San Diego, Calif.) [8] for direct immunofluorescence. Incubation was carried out for 30 min at 4 °C followed by extensive washing. For indirect immunofluorescence, free binding sites of the secondary antibody were blocked with 10% mouse serum. The cells were analyzed in a FACScan (Becton Dickinson, San Jose, Calif.), excluding nonviable cells.

ELISA

For detection of anti-mouse-R 73 antibodies, microtiter plates were coated with 1.5 µg R 73 per well. Sera of animals collected before and after grafting, mouse $F(ab')_2$ anti-rat-IgG-AP (50 µl, dilution 1:5000, Jackson Immuno Research Laboratory, West Grove, Pa.), and PNP substrate were added sequentially. All incubations were performed at room temperature for 1 h and followed by extensive washing with PBS tween 20 (0.05%). Optical density was measured in an ELISA-reader (SLT Laboratories, Crailsheim, Germany) at 405 nm.

Statistics

Results are expressed as mean $\pm$ SD. Student's t-test was used for comparison of means and allograft survival rates were compared by the Wilcoxon rank sum test. A statistical difference of $P < 0.01$ was considered significant.

Results

Prolongation of cardiac allograft survival

The graft survival time in the control animals was 7 ± 1 days. Short-term R 73 treatment in young adult recipients (Table 1; group I a) prolonged graft survival up to 62 days

Table 1 Survival times of cardiac allografts after R 73 therapy

Treatment protocol	Graft survival times (days)	Significance
Group Ia Short-term R 73 treatment in 6-week old recipients	30, 30, 32, 51, 62, 108, > 500, > 500, > 500	$P < 0.0001$
Group Ib Short-term R 73 treatment in 10-week old recipients	12, 12, 13, 16, 17, 19, 19, 21, 21, 21, 21, 21, 26, 31	$P < 0.00001$
Group IIa Chronic R 73 therapy in newborns until transplantation	22, 31, 31, 36, 38, 41, 42, 54, > 200, > 200	$P < 0.0001$
Group IIb Chronic R 73 therapy in newborns to day 70 after transplantation	105, 150, 153, 153, > 200, > 200, > 200, > 200, > 350, > 350	$P < 0.0001$
Group III No treatment	6, 6, 7, 7, 7, 7, 7, 7, 8, 9	

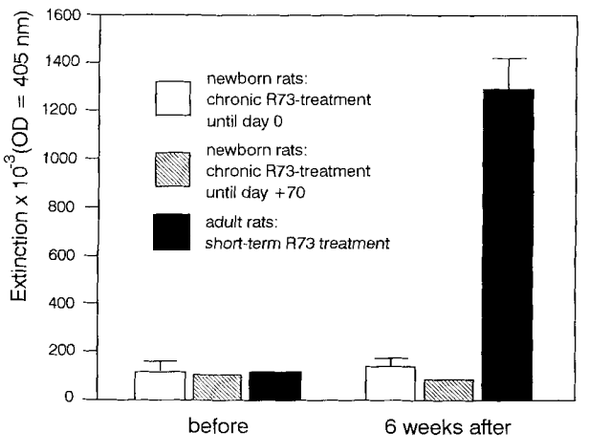

Fig. 1 Anti-R 73 antibody titer in sera of rats before and 6 weeks after transplantation

in five of nine animals. The remaining four animals accepted their grafts for more than 100 days. Older adult animals (group I b) receiving the same treatment rejected their grafts within 31 days. Chronic postnatal treatment up to the time of transplantation (group II a) resulted in graft survival for 37 ± 9 days in eight of ten animals. The remaining two animals accepted their grafts for more than 200 days. All grafts were accepted for more than 100 days when postnatal R 73 therapy was continued to day 70 after grafting (group II b).

Elimination of α/β T-cells

The first injection of R 73 in all adult animals reduced the percentage of α/β T cells from $69 \pm 3\%$ to $5 \pm 2\%$. However, 10 days later, α/β T cells returned to pretreatment levels ($64 \pm 6\%$) in spite of continued R 73 therapy. Chronic R 73 therapy in newborns caused permanent depletion of α/β T cells below 1% during the course of administration. After the therapy was stopped, a gradual increase in α/β T cells to 33% on day 20, 44% on day 50, and 63% on day 200 was noted. In two animals, R 73-positive cells remained below 10% for more than 150 days after the end of treatment.

Anti-R 73 antibodies

The return of α/β T cells in all short-term treated adult animals was paralleled by the appearance of antibodies directed against R 73 (extinction: before treatment = 103 ± 11; day $5 = 698 \pm 100$, day $9 = 1408 \pm 303$, and after 6 weeks $= 1300 \pm 120$). All chronically treated newborn animals tolerated the mouse immunoglobulin and no anti-R 73 antibodies were detected (extinction < 170; Fig. 1).

Discussion

Selective elimination of α/β T cells with the monoclonal antibody R 73 induced long-lasting rat cardiac allograft survival in chronically treated newborn Lewis recipients. Animals treated from birth were tolerant to foreign mouse immunoglobulin and did not form anti-R 73 antibodies. In these animals, the efficacy of R 73 was not impaired and α/β T cells were eliminated during the whole course of therapy. Since γ/δ T cells were not affected by R 73, we speculated that γ/δ T cells themselves or without α/β T cell help are not able to cause allograft rejection. Treatment of adult animals with R 73 was paralleled by the appearance of anti-R 73 antibodies within the first week. This explains why the percentage of α/β T cells decreased temporarily and reached normal levels after 10 days. With short-term administration, the graft-protective effect of R 73 was stronger in younger than in older adult animals. Three out of nine young animals accepted their grafts for more than 500 days. This difference may be due to an age-dependent delay in the seeding of lymphatic organs by T cells [9] or to the lack of a costimulatory pathway and a deficit in interleukin-2 production [10, 11].

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